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QUANTITATIVE COMPARISON OF THE CYTOGENETIC EFFECT OF THIOPHOSPHAMIDE ON MONKEY LYMPHOCYTES IN VIVO AND IN VITRO

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Before regulations governing the use of chemicals with mutagenic activity can be drawn up, their mutagenic effect must be quantified. One approach to the solution of this problem is to establish the principles of extrapolation of data obtained during testing of chemicals on mutagenicity in vitro to the intact organism. It was shown previously that in the case of the action of thiophosphamide on rabbit lymphocytes [1, 3] and of cyclophosphamide on human lymphocytes in vitro and in vivo similar dose–effect relationships are observed during analysis of sister chromatid exchanges (SCE) and of chromosomal aberrations (CA) provided that certain principles of the conduct of chemical dosimetry and evaluation of the effects are observed.

The aim of this investigation was to make a quantitative comparison of induction of SCE and CA during the action of thiophosphamide on monkey lymphocytes in vivo and in vitro.

EXPERIMENTAL METHOD

Experiments were carried out on three sexually mature male rhesus monkeys. Before the animals were treated with cyclophosphamide samples of 8 ml of blood were taken for the control tests and for the experiments in vitro. Thiophosphamide, diluted in 4 ml of distilled water, was injected intravenously in a dose of 3 mg/kg body weight. During the 4 h after injection of the compound blood was taken several times from a vein in a volume of 3-4 ml, and part of it was used to determine the mutagenic effect in vivo, the rest to determine the thiophosphamide concentration in the nitrobenzylpyridine test by the method described previously [2].

In experiments in vitro 8.5 ml of Hanks' solution with thiophosphamide in a final concentration of 2 to 15 μg/ml, in different versions of the experiments, were added to 1.5 ml of blood. The mixture was incubated for 1 h at 37°C.

In the experiments in vivo and in vitro the lymphocytes were washed 3 times with 10 volumes of Hanks' solution to remove the mutagen. The cells were cultured in the usual way for 73 h with 5-bromodeoxyuridine, which was added at the beginning of culture in a final concentration of 10 μg/ml. Concanavalin A (Sigma, USA) in a final concentration of 15 μg/ml was used as the mitogen.

CA were counted in the preparation in the first mitoses, and SCE in the second mitoses, during anlaysis of 2500 and 600 metaphases, respectively.

To quantify the mutagenic effect the dose of mutagenic action (D) was determined by the method described previously [1] as the integral of the function of the change in thiophos-

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TABLE 1. Frequency of CA and SCE during Action of Thiophosphamide on Monkey Lymphocytes in Vivo and in Vitro

Monkeys	Time after injection, min	Dose, μg·min/ml	Chromosomal aberrations		Number of SCE per cell (M ± m)
			fraction of aberrant metaphases	number of breaks per metaphase	
		In vivo			
1	Before injection	0	0,03	0,03	4,68±0,38
	35	225	0,11	0,14	13,56±0,83
	65	405	0,17	0,21	28,32±1,05
	130	756	0,27	0,37	41,08±2,08
2	Before injection	0	0,03	0,03	4,60±0,39
	30	221	0,09	0,10	14,36±0,57
	60	427	0,14	0,17	25,60±0,71
	120	796	0,26	0,35	38,08±0,98
	210	1258	0,38	0,69	51,50±1,30
3	Before injection	0	0,03	0,03	5,36±0,45
	30	277	0,04	0,04	17,72±1,10
	75	613	0,09	0,11	35,36±1,09
	155	1034	0,20	0,34	53,16±1,29
	230	1287	0,35	0,65	69,88±1,64
		In vitro			
1	—	0	0,03	0,03	4,68±0,38
		150	0,08	0,08	10,32±0,50
		375	0,14	0,17	19,16±0,71
		750	0,24	0,39	37,00±1,48
		1125	0,31	0,53	43,00±1,04
2	—	0	0,03	0,03	4,60±0,39
		150	0,04	0,05	8,88±0,48
		375	0,13	0,21	17,12±0,69
		750	0,25	0,40	30,76±1,04
		1125	0,35	0,51	44,64±1,26
3	—	0	0,03	0,03	5,36±0,45
		300	0,10	0,17	28,68±1,22
		750	0,28	0,58	62,00±1,93
		1125	0,46	0,93	72,80±2,13

phamide concentration with time: in the experiment in vivo from the time of its injection into the animal to the time when the blood sample was taken; in the experiment in vitro, during incubation of the cells until replacement of the solution with mutagen by fresh Hanks' solution.

EXPERIMENTAL RESULTS

Data showing changes in the frequencies of CA and SCE in monkey lymphocytes at different times after injection of thiophosphamide in vivo and during a change in its concentration in vitro are given in Table 1. Clearly the cytogenetic effect increased with an increase in the concentration or duration of action of thiophosphamide and, correspondingly, with an increase in the dose of mutagenic action.

To describe dependence of the frequencies of CA and SCE on D regression analysis was carried out, using the equations suggested previously: for the fraction of cells with CA, $\rho = 1 - e^{-(a+kD)^2}$; for the number of chromosomal breaks per cell $X = e^{(a+kD)^2} - 1$ [4]; for the number of SCE per cell $S = b_0 + b_1 D$ [3], where a, k, b_0, and b_1 are regression coefficients. The analysis showed that these equations satisfactorily describe the experimental data obtained both in vivo and in vitro. Thus the dose–effect relationship was similar in character in the two test systems. Regression coefficients also were similar both in each animal and when data for three animals were pooled, when experimental results in vivo and in vitro were compared. Values of the coefficients k and b_1, reflecting the effectiveness of thiophosphamide in inducing CA and SCE, was $k = 3.69 \cdot 10^{-4} \pm 0.96 \cdot 10^{-4}$ for the fraction of aberrant metaphases, $k = 4.06 \cdot 10^{-4} \pm 0.88 \cdot 10^{-4}$ for the number of chromosomal breaks per cell; and $b_1 = 0.0445 \pm 0.0055$ for the number of SCE per cell in the experiments in vivo; the corresponding figures for the experiments in vitro were $4.56 \cdot 10^{-4} \pm 0.64 \cdot 10^{-4}$, $4.82 \cdot 10^{-4} \pm 0.88 \cdot 10^{-4}$, and 0.0450 ± 0.0147.

To approach to quantitative evaluation of the mutagenic effect used in this investigation enables the two principal parameters influencing this effect to be obtained. These are the dose, i.e., the degree of mutagenic action on the cells, and the sensitivity of the

cells to the mutagen, described by regression coefficients reflecting the dependence of effect on dose. Determination of the value of D, which takes into account both the duration of action and the change in concentration of the mutagen, enables the results of testing the mutagens in different test systems to be compared adequately, regardless of the quantity of the substance used or the length of its exposure.

The results, like those of previous investigations on rabbit and human lymphocytes [1, 3, 5], indicate that the sensitivity of the cell to the mutagen is similar in vivo and in vitro. Quantitative evaluation of the mutagenic effect in vivo is thus possible by extrapolating the results obtained by testing a chemical in vitro and determining the dose of mutagenic action in vivo.

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